STUDIES ON MYXOBACTERIA

WITH SPECIAL REFERENCE TO GROWTH CONDITIONS AND BACTERIOLYTIC ACTIVITY

BY

BÖRJE NORÉN

UPPSALA 1955

ALMQVIST & WIKSELLS BOKTRYCKERI AB

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FIL. LIC., V.-D.

INAUGURAL DISSERTATION
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
WHICH BY DUE PERMISSION OF THE PHILOSOPHICAL
FACULTY OF THE UNIVERSITY OF UPPSALA WILL
BE PUBLICLY DEFENDED IN THE BOTANICAL AUDITORIUM,
ON MAY 18TH, 1955, AT 10.00 A.M.
IN ADDITION TO THIS SURVEY THE PAPERS I–VII IN THE LIST
OF REFERENCES GIVEN IN THE INTRODUCTION
WILL BE PUBLICLY DEFENDED

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Contents

Page

Preface 4
Introduction 5
A. Distribution of Myxobacteria in Soils 6
1. Myxobacteria found in Swedish soils 6
2. Factors influencing the occurrence in soil 7
B. Growth Conditions 8
1. Material and methods 8
2. Composition of the basic media 8
a. Concentration of casein hydrolysate 8
b. Influence of calcium, iron, manganese, and zinc 9
c. pH relations 9
3. Influence of temperature 10
4. Utilization of carbohydrates 11
5. Relations to amino acids 12
6. Vitamin requirements 13
7. Relations to nucleic acids and nucleic acid components 14
C. Bacteriolytic activity 15
1. Lytic acitivity of myxobacteria in a suspension of eubacteria ... 15
2. Factors influencing the bacteriolysis 15
a. The myxobacterium employed 15
b. The eubacterium employed 16
c. Cultural conditions 16
3. Observations on the bacteriolytic substances 17
D. Production of Antibiotics 17
References 18

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Preface

The present investigation has been carried out at the Institute of Physiological Botany, University of Uppsala. I express my sincere gratitude to its Founder and Director, Professor Elias Melin, not only for the lively interest he has accorded my work, but also for the benefit of encouraging discussions and for all the support he has so kindly given me in every respect.

Sincere thanks also go to my other teachers in botany, Professor John Axel Nannfeldt and Professor G. Einar Du Rietz, for the valuable instruction and advice I have received from them during my university years.

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Finally, I should like to thank my wife, Mrs. Thyra Norén, Fil. mag., whose constant encouragement and unflagging interest have greatly stimulated me during the course of this work.

Uppsala, Institute of Physiological Botany
March 1955

Börje Norén

Introduction

This paper is a summary of the following communications by the author:

I. Further notes on the distribution of myxobacteria in Swedish soils. Svensk Bot. Tidskr. 46: 446–453, 1952.
II. Myxobacteria in soils. Nature 167: 225–227, 1951.
III. Studies on myxobacteria. I. Growth conditions. Svensk Bot. Tidskr. 46: 324–365, 1952.
IV. Studies on myxobacteria. III. Organic factors in nutrition. Bot. Not. 108: 81–134, 1955.
V. Studies on myxobacteria. II. Bacteriolytic activity. Svensk Bot. Tidskr. 47: 309–332, 1953.
VI. Studies on myxobacteria. IV. Lytic activity on different eubacteria. Svensk Bot. Tidskr. 49: 282–294, 1955.
VII. On the production of antibiotics by myxobacteria. Svensk Bot. Tidskr. 47: 402–410, 1953.

In the following, references to these papers will be indicated by means of the Roman numerals I–VII.

When the experimental studies underlying the present paper were begun in 1949 the first purpose was to study the capability of myxobacteria to cause lysis of various eubacteria. *Myxococcus virescens*, *Myxococcus fulvus* and *Chondrococcus coralloides*, which were found to be wide-spread in nature, were chosen as test organisms. A prerequisite for such a study is that the principal features of the general physiology of the organisms tested are known. It soon became evident, however, that information about the general physiology of the myxobacteria in question was actually very incomplete and in some cases the data recorded in the literature were contradictory. Therefore experiments were carried out with the main intention to compose a liquid medium which provides good—if possible, optimum—conditions for the myxobacterial growth. However, this task met with a large number of problems that had to be elucidated, viz. the utilization of carbohydrates, the relations to amino acids, the influence of temperature and pH, the effect of certain metallic salts, of vitamins and nucleic acids. As a result, the study became more comprehensive than originally planned, and it became necessary to

concentrate the work on the one species *Myxococcus virescens*. Simultaneously with the investigation into the growth conditions of myxobacteria some experiments on their bacteriolytic activity were carried out. In order to obtain a more detailed information about the myxobacterial activity the lysis occurring in autoclaved suspensions of eubacteria was studied and some factors influencing this process elucidated. In connection, some tests on the production of antibiotics were performed.

The pure cultures used in the physiological experiments were isolated from soils. The isolation experiments were arranged in such a way that information was obtained on the distribution of myxobacteria in nature and on certain factors influencing their occurrence.

A. Distribution of Myxobacteria in Soils (I, II)

The fact that myxobacteria are common soil inhabitants was first demonstrated by the KRZEMIENIEWSKIS (1927) and their finding has been verified by all subsequent investigations.

Several methods of obtaining myxobacteria from soils are given in the literature (KRZEMIENIEWSKI 1927, KONONENKO 1937, SOLNTZEVA 1939b, BEEBE 1941a, SINGH 1947). In the present investigation those of KRZEMIENIEWSKI (1927) as modified by BEEBE (1941a), and SINGH (1947) were followed.

Some experiments performed have shown that the KRZEMIENIEWSKI-BEEBE method does not give comparable data corcerning the distribution of myxobacteria in different types of soils, since the rabbit dung pellets used in this technique do not constitute a constant medium. SINGH's method has the advantage that myxobacteria have to develop on a standardized medium but, on the other hand, only bacteriolytic myxobacteria can be obtained in this way (II).

Pure cultures of myxobacteria were obtained by the methods of BEEBE (1941a) and SINGH (1947). The last one was found to be the most rapid and reliable (I).

1. Myxobacteria found in Swedish soils (I)

In the soils of Central Sweden almost every sample examined was found to contain myxobacteria. The following species were

identified: *Archangium gephyra* JAHN, *Archangium primigenium* JAHN, *Podangium erectum* JAHN, *Melittangium boletus* JAHN, *Myxococcus fulvus* JAHN, *Myxococcus virescens* THAXTER, *Myxococcus xanthus* BEEBE, and *Chondrococcus coralloides* JAHN. Fruiting bodies apparently belonging to *Polyangium fuscum* THAXTER were also observed. *Archangium* sp., *M. fulvus, M. virescens* and *Ch. coralloides* were found to be the most common species.

In the soils of the alpine region of Abisko, northern Sweden, where myxobacteria were found more rarely, *Archangium* sp., *M. fulvus, M. virescens,* and *Ch. coralloides* were isolated. *M. fulvus* was the most frequent species.

It should be noted that *M. virescens* was also found in a soil sample from Spitzbergen.

2. Factors influencing the occurrence in soil (I, II)

The numerous isolation experiments performed have revealed that certain variations exist in the myxobacterial frequency in different types of soils. Myxobacteria occur in large numbers in composts and well-kept garden soils, whereas they are few in the acid soils of forests. In fields they are always numerous. As mentioned before, in soils of the alpine region of Abisko myxobacteria occur only sparingly (cf. KRZEMIENIEWSKI 1927).

In some experiments it was demonstrated that *M. virescens* is most frequent in neutral and alkaline soils. *M. fulvus* shows the same tendency, while *Ch. coralloides* seems to be less numerous in the alkaline soils (cf. KRZEMIENIEWSKI 1927, SINGH 1947). The tendencies in pH relation that appear from these results have been confirmed by data obtained in physiological experiments with pure cultures (see below).

As could be expected the soil treatment influences the occurrence of myxobacteria in soils. It is, however, an interesting fact that a soil that has been left untreated for more than 100 years seems to contain the same number of *M. virescens, M. fulvus* and *Ch. coralloides* as does a soil that has received a complete mineral treatment during the same period. On the other hand, treatment with farmyard manure greatly increases the number myxobacteria present.

For the distribution of myxobacteria in the alpine region the summer temperature seems to be the determining factor. Conditions of intense insolation resulting in strongly heated soils in the summer

favour the occurrence of at least *M. fulvus*. As regards *M. virescens*, however, a certain tendency to a concentration in particularly fertile soils is evident.

B. Growth Conditions (III, IV)

1. Material and methods (III, IV)

In the present investigation, three myxobacteria were mainly employed: *Myxococcus virescens*, *M. fulvus*, and *Chondrococcus coralloides*. *Archangium primigenium* and *Myxococcus xanthus* were tested in two and one experiments respectively.

With few exceptions the experiments were carried out in liquid media in culture tubes, these being mechanically shaken during the incubation. As inoculum a suspension of myxobacteria in 0.9% NaCl, containing both vegetative cells and microcysts, was used.

The development of the myxobacterial pseudoplasmodia appearing on the glass walls of the tubes was followed photometrically, the extinction being determined in white light by means of the photometer used by Åberg and Rodhe (1942) and the various *e*-values calculated according to the formula $\log \frac{I_0}{I}$. In agreement with Rodhe (1948) the differences in extinction are expressed in the formula $Z = (e_v - e_0) \cdot 10^3$. The increase of Z caused by a developing pseudoplasmodium was taken as a measure of the growth of the myxobacteria.

2. Composition of the basic media (III)

a. *Concentration of casein hydrolysate*

The present investigation started from the finding by Oxford (1947) that *M. virescens* can grow in a nutrient solution containing casein, hydrolyzed by acid to the amino acid stage, as the sole source of carbon, nitrogen and energy. A test of various concentrations of casein hydrolysate showed that the best growth occurred at 0.25 %. A concentration of 0.5 % caused growth inhibition during the first phase of growth but later, when the amino acids had been partly consumed by the myxobacteria or when the organisms had become adapted to the medium, vigorous growth took place.

b. *Influence of calcium, iron, manganese, and zinc*

The compounds calcium chloride and ferric citrate are both found to have a twofold effect on *M. virescens*: (1) The growth is greatly improved, the improvement being primarily visible in a more rapid development. (2) The pigmentation of the fruiting bodies is strongly affected so that by increasing the Ca or Fe content of the medium the fruiting bodies become greener. The effects must evidently be attributed to the Ca ion and the Fe ion.

The growth of *M. fulvus* is also stimulated by the addition of Ca or Fe, while in the case of *Ch. coralloides* the differences obtained were too small to warrant any definite conclusion.

The addition of a manganese or zinc salt gave rise to higher maximum values of *Z* in *M. virescens* but the standard errors were too great to allow any definite conclusion. In the case of *M. fulvus* and *Ch. coralloides* Mn produced certain positive effects while Zn had no detectable influence.

c. *pH relations*

Since previous records on the pH relations of myxobacteria are somewhat conflicting (Krzemieniewski 1927, Solntzeva 1940, Beebe 1943, Singh 1947, Oxford 1947), some experiments were carried out to study this subject.

In a casein hydrolysate medium the growth of *M. virescens* is greatly influenced by the hydrogen-ion concentration of the medium. A marked optimum appears at pH 7.9 (cf. Solntzeva 1940). From this optimum the growth gets rapidly weaker towards the acid side and no growth occurs at a pH below 7.0. On the other hand, a certain but very weak growth is produced at pH 9.4. *M. fulvus* seems to have pH relations similar to *M. virescens*. Contrary to these two myxobacteria, *Ch. coralloides* appears to thrive best with a pH of about 6.5. However, this organism formed thin but coherent pseudoplasmodia at pH values varying from 4.9 to 8.7.

The results obtained correspond fairly well with the observations made on the distribution in soils of these organisms (H, Krzemieniewski 1927). Singh (1947), however, did not find any correlation between the pH of the soils and the distribution of species.

On the basis of the results obtained with the three myxobacteria tested the basic nutrient solution was given the following composi-

tion: casein hydrolysate 2.5 g, asparagine 2.5 g, K_2HPO_4 2.0 g, NaCl 1.0 g, $MgSO_4 \cdot 7H_2O$ 0.1 g, $CaCl_2$ 10.0 mg, $MnSO_4 \cdot 4H_2O$ 1.0 mg, ferric citrate 3.0 mg, and distilled water 1000 ml. For *M. virescens* and *M. fulvus* the pH of the medium was adjusted to 7.5. Owing to the ammonia formation this pH will shift in an alkaline direction during incubation. Owing to this fact an initial pH value of 7.5 was chosen in preference to the somewhat higher optimum value 7.9. If the later value were used the substratum would too soon reach a pH unsuitable to luxuriant growth. In the case of *Ch. coralloides* the pH was adjusted to about 6.5

The above nutrient solution is a good medium for *M. virescens*. On the submerged walls of the culture tubes the pseudoplasmodia appear as thick films. It was evident, however, that the casein hydrolysate could by no means be considered as an ideal source of carbon, nitrogen and energy and no optimum growth was produced. *M. fulvus* does not grow as satisfactorily as *M. virescens*, but thick pseudoplasmodial films are formed on the submerged tube walls. The growth of *Ch. coralloides* in this medium is poor, but coherent pseudoplasmodial films are always produced.

3. Influence of temperature (III)

Literature contains some records on the influence of temperature on the growth of myxobacteria (Baur 1905, Quehl 1906, Kofler 1913, Krzemieniewski 1927, Solntzeva 1940). The present investigation was carried out on an agar medium since it was not feasible to perform it in a liquid medium.

The vegetative development of *M. virescens* and *M. fulvus* is best at a temperature of 32°–34°C, whereas a temperature of 34°–36°C seems to be most favourable for the formation of fruiting bodies. A certain growth occurs at 40°C. The lower limit for growth of both the organisms appears to be somewhere below 15°C.

Optimum growth of *A. primigenium* occurs at 33°C and this species grows at 19°C and at 40°C. Fruiting bodies are developed most frequently at 33°C.

Ch. coralloides grows best at a temperature between 25°C and 30°C. A good development occurs at 15°C. At 36°C the growth is totally inhibited. The greatest number of fruiting bodies was found at 25°C.

4. Utilization of carbohydrates (IV)

The investigation has revealed that *M. virescens* is able to utilize starch and glycogen and their hydrolytic products dextrin and maltose, and these compounds are available as sources of carbon and energy. Several previous investigations have pointed out that starch is a suitable carbon source for the organism in question (Jahn 1939, Solntzeva 1940, Beebe 1943, Kühlwein 1950, Oetker 1953, Clark 1954). As regards the remaining three carbohydrates no effects or negative effects have been registered (Solntzeva 1940, Beebe 1943, Clark 1954), which may be due to the use of unsuitable culture methods. On the other hand, in *M. fulvus* positive results were obtained with maltose (Solntzeva 1940, Kühlwein 1950, Clark 1954) and glycogen (Solntzeva 1940).

In a very low concentration glucose slightly improves the growth, but at higher concentrations no effects or negative effects are produced (cf. Clark 1954). These observations combined with the results of previous investigations (Baur 1905, Finck 1950, Oetker 1953) seem to indicate that the organism is able to utilize this carbohydrate, though the uptake occurs but slowly. However, this view is not in accordance with the finding of Solntzeva (1940) that no consumption of glucose occurred in a medium containing peptone in spite of good growth of *M. virescens.*

Arabinose, galactose, sucrose, and raffinose are also available to *M. virescens* as a source of energy and carbon but they improve the growth to a lesser extent than do starch and compounds related to starch. According to the records by Solntzeva (1940), Beebe (1943), Kühlwein (1950), and Clark (1954), none of these sugars should be utilizable by *M. virescens.* However, Yoshii (1926) found that this organism grew well in a peptone-sucrose solution and as regards *M. fulvus* there are some observations on positive effects on the growth by these sugars (Solntzeva 1940, Kühlwein 1950, Clark 1954).

The presence of xylose, ribose, fructose, mannose, lactose, cellobiose or inulin in the medium does not improve the growth of *M. virescens.* These results agree with the previous findings by Solntzeva (1940), Kühlwein (1950), and Clark (1954). However, a certain influence on the pH changes during the incubation is evident.

The growth of *M. fulvus* is not influenced by the addition of ribose, while in the case of *Ch. coralloides* a slightly positive effect is produced.

5. Relations to amino acids (III, IV)

As mentioned above, casein hydrolysate provides a more or less good growth of *M. virescens, M. fulvus,* and *Ch. coralloides.* An addition of asparagine has a certain effect on the growth of *M. virescens* and *M. fulvus* but does not seem to influence that of *Ch. coralloides.* An addition of tryptophane improves the growth of *M. fulvus* (III).

Experiments performed have revealed that casein hydrolysate provides a more vigorous growth of the three myxobacteria tested than does a mixture of amino acids, used in the same proportions as those found in the complete casein hydrolysate. With each of the 19 various amino acids of the mixture *M. virescens* forms pseudoplasmodia, as does *M. fulvus* except with glycine (cf. Kühlwein 1950) and alanine. Both species grew best with leucine, glutamic acid, methionine and arginine. In no case was an optimum or even a good growth obtained, possibly owing to the fact that the amino acids were used only in low concentrations (III). In higher concentrations at least some of them may be inhibitory (Solntzeva 1940). It is a noticeable fact, however, that Finck (1950) found only aspartic acid—and β-alanine—available to *M. virescens,* while glutamic acid, leucine, and *l*-cystine were inhibitory though they were tested in a low concentration.

A more detailed study was carried out with *M. virescens* as the test organism (IV). Two series of experiments were set up.

A nutrient solution containing vitamin-free casein hydrolysate in a concentration of 1 g/l was supplemented by each of the 19 amino acids of a complete casein hydrolysate in turn. The proportions in which the amino acids were used were the same as are found in casein hydrolysate and calculated on the basis of 1 g of the latter. It was found that an addition of valine, phenylalanine, glutamic acid, proline or arginine greatly improved the growth, the effect being obvious both as an increased rate of growth and an increased total production of living matter. Aspartic acid, threonine, cystine and methionine produced only the latter effect. Serine and isoleucine had a weaker effect and they formed a transition to the next group of amino acids, comprising glycine (cf. Oxford 1947), leucine, tyrosine, tryptophane, hydroxyproline and histidine, which did not affect the growth in any detectable way. Alanine (cf. Oxford 1947) very slightly increased the rate of growth, while lysine was slightly inhibitory to the initial phase of growth (cf. Oxford 1947).

In other experiments an amino acid mixture corresponding to a complete casein hydrolysate was used as the sole source of carbon, nitrogen, and energy. This mixture was modified in successive cases by the removal of one of the amino acids. An omission of glycine, valine, leucine or isoleucine caused an almost total growth inhibition. A less marked inhibition was obtained when glutamic acid, tyrosine, methionine or arginine was removed from the mixture. On the other hand, the somewhat surprising observation was made that a mixture deprived of alanine, tryptophane, aspartic acid, proline, hydroxyproline, serine, threonine, histidine or lysine produced a better growth than the complete amino acid mixture. The omission of alanine or histidine had a particularly growth-stimulative effect.

The results obtained in these experiments seem to indicate that for the growth of *M. virescens* glycine, valine, leucine and isoleucine are necessary components of the mixture of amino acids. Glutamic acid, methionine, and arginine and also possibly tyrosine are apparently stimulative elements.

The results also make it probable that the amino acids in a mixture corresponding to a complete casein hydrolysate do not occur in the proportions suitable for an optimum growth of *M. virescens*. However, the lack of balance may be at least reduced by an omission of alanine, histidine, lysine, tryptophane, aspartic acid, hydroxyproline, proline, threonine or serine or by an addition of certain amounts of phenylalanine, proline, aspartic acid, threonine or cystine.

6. Vitamin requirements (III, IV)

The investigation has revealed that *M. virescens* can produce growth in a completely vitamin-free casein hydrolysate medium (IV). This means that the organism is capable of carrying out the synthesis of all the vitamins which are necessary for growth. However, an addition of riboflavin, Ca-pantothenate, folic acid, biotin and choline, separately or as a mixture, has a stimulating effect on the growth. Thus, under the given experimental conditions the organism synthesizes these vitamins only at such a slow rate that they act as limiting factors for growth.

The degree of the partial vitamin deficiency seems to be greatest for choline, which in the concentration of 1000 μg/l greatly increases both the rate and the total yield of growth. As regards micro-

organisms, this vitamin has so far been shown to be growth-stimulative only in a few cases (IV).

The remaining stimulative vitamins act chiefly on the rate of growth, though riboflavine and biotin show slight tendencies to increase the amounts of growth as well (IV). All these vitamins are active in considerably lower concentrations than choline. An optimum effect appears to be reached with 200 µg/l riboflavine, 100 µg/l Ca-pantothenate, 100 to 200 µg/l folic acid and 100 mµg/l biotin. From the results obtained it is evident that the organism reacts rather sensitively to an excess of the vitamins. Already doses doubled as compared with the above, are more or less inhibitory. This sensitivity has possibly influenced the results obtained by Oetker (1953).

When grown in a medium containing non-vitamin-free casein hydrolysate *M. fulvus* is markedly stimulated by an addition of folic acid and choline (III). It is not yet known if both or only one of the substances are growth-stimulative. As regards *Ch. coralloides* the experiments performed have not revealed any conclusive data concerning the vitamin requirements.

7. Relations to nucleic acids and nucleic acid components (IV)

M. virescens, *M. fulvus*, and *Ch. coralloides* are capable of utilizing RNA, adenosine, guanosine, cytidine, thymine and, to a lesser extent, DNA as sole sources of carbon, nitrogen, and energy.

When added to a medium with non-vitamin-free casein hydrolysate, RNA greatly improves the initial growth of *M. virescens*. A stimulative effect similar to that of RNA is also produced by adenosine. An addition of 0.1 g/l of RNA or adenosine to a medium containing 1.25 g/l casein hydrolysate improves the initial growth as much as does an addition of 1.25 g/l casein hydrolysate, but the latter gives a higher total yield of growth. Cytidine and thymine stimulate the initial phase of growth somewhat less but increase the rate of the consequent development somewhat more than adenosine. DNA and guanosine have an inhibitory influence on growth.

When cultured in a casein hydrolysate medium *Ch. coralloides* is only slightly affected by an addition of RNA, adenosine, guanosine, cytidine, or thymine and no differences in their effect are detectable. On the other hand, in the case of DNA an inhibitory influence is evident.

C. Bacteriolytic Activity (V, VI)

The capability of myxobacteria to produce lysis of eubacteria was first observed by Pinoy (1913). In his investigation as in all the following ones, the experiments were carried out upon a solid medium, the eubacterial cells being either suspended in the medium or spread over the surface. Owing to their lytic activity, the myxobacteria caused clear zones in the dense masses of true bacteria. It is evident that such a method cannot permit a close study of the bacteriolysis, since it is impossible to obtain exact data on its course and intensity.

In the present investigation the lytic activity of myxobacteria has been tested in autoclaved eubacterial suspensions, the clearing of which was followed photometrically. The decrease in extinction of a eubacterial suspension has been taken as a measure of the bacteriolytic effect (V).

1. Lytic activity of myxobacteria in a suspension of eubacteria (V)

The first experiments performed examined the bacteriolysis occurring in a suspension of *Staphylococcus aureus* in distilled water. If a myxobacterial liquid culture is used as inoculum a bacteriolysis comprising three phases is produced: (1) An initial, short period with a heavy lysis, caused by the bacteriolytic substances present in the inoculum liquid, (2) a more or less stagnant period, and (3) a new period with heavy lysis, caused by the bacteriolytic substances produced by growing myxobacteria. If a small quantity of a cell-free culture solution is used as inoculum only the first phase is produced, i.e. the bacteriolysis is very intense during the first hours but decreases later. It is thus evident that the activity of the bacteriolytic substances greatly decreases after only a short time. An inoculum containing only microcysts gives rise only to the 3rd phase, which fact indicates that only the vegetative cells are lytically active while the microcysts are not.

2. Factors influencing the bacteriolysis (V, VI)

a. *The myxobacterium employed*

Some experiments showed that the intensity of the bacteriolysis can vary with the myxobacterium employed. This observation agrees

with some previous observations (Solntzeva 1939a, Beebe 1941b, Singh 1947) and it is confirmed by the record by Oetker (1953). However, although the intensity differs, the lysis produced by *M. virescens*, *M. fulvus* and *Ch. coralloides* in a suspension of eubacterial cells appears to follow the same lines, and no fundamental differences seem to exist.

b. *The eubacterium employed*

Experiments performed on the lytic activity of myxobacteria on different eubacteria have confirmed the findings by Solntzeva (1939a), Beebe (1941b), Singh (1947) Imšenetskij & Kusjurina (1951), and Oetker (1953) that different eubacteria vary in their resistibility to a myxobacterial attack. It is found that the resistibility can vary even from one species to another within the same genus. As yet the factors which control the eubacterial resistance are not known. Some results which have been recorded by Beebe (1941b) and Singh (1947) seem to indicate that Gram-positive eubacteria are more resistent than Gram-negative, and pigmented more than non-pigmented, but in my experiments the Gram-positive *Staphylococcus aureus* and the pigmented *Serratia marcescens* were more rapidly lysed than the Gram-negative and non-pigmented *Escherichia coli*.

In view of all the previous findings (Solntzeva 1939a, Snieszko *et al.* 1942, Beebe 1941b, Singh 1947, Oetker 1953) it is not surprising that the myxobacteria tested produce growth in a suspension of eubacterial cells in distilled water. It is, however, a noticeable fact that good growth occurs even if the myxobacteria are capable of only a very slow lysis of the eubacterium employed.

c. *Cultural conditions*

Substratum (VI).—The results obtained have emphasized the fact that the composition of the medium is an important factor for the bacteriolytic activity of myxobacteria. A eubacterial species or strain which is not at all or only slightly lysed in one medium may be readily attacked and lysed in another richer medium.

The experiments have revealed that substances such as casein hydrolysate, $CaCl_2$ and ferric citrate increase the rate of the bacteriolysis caused by *M. virescens*. However, these compounds also favour the multiplication of the myxobacteria and it is not yet clear if the

increased lytic intensity results from this. Some facts seem to indicate that Nutrient solution III B (IV, p. 82) contains some factor(s) that stimulate the production of bacteriolytic substances per cell or —and?—increase the effect of these substances.

The experiments have also shown that pure cultures of myxobacteria produce and secrete bacteriolytic substances even if no eubacteria are present.

pH (V).—A culture solution in which *M. virescens* has been grown has a slight but definite bacteriolytic effect at a pH of 4.1 and still at 9.6 a pronounced lysis occurs. Thus, the bacteriolytic substances produced by this organism are active within a fairly wide pH range. The optimum is about 7.3. However, the lysis caused by growing cells of *M. virescens* is more intense at a somewhat higher pH since the optimum pH value for the growth of this myxobacterium lies at pH 7.9 (III). At a too low pH value the cells do not produce any lysis.

3. Observations on the bacteriolytic substances (VI)

The experiments have revealed that in *M. virescens* and *Ch. coralloides* the bacteriolytic activity results from two lytically active systems (cf. Oetker 1953). System A produces a rapid lysis of the used strains of *Staphylococcus aureus, Aerobacter* sp. 1912, and *Serratia marcescens* while it is less active in the cases of *Aerobacter aerogenes, Escherichia coli,* and *Bacillus subtilis.* It is questionable if it is capable of a complete disintegration of the eubacterial cells. System B may be lytically active on all the eubacteria tested and it seems to produce a complete lysis of the cells.

Two-day-old liquid cultures of *M. virescens* and 4-day-old cultures of *Ch. coralloides* contain both System A and System B while 10-day-old cultures are apparently lacking in System B. Thus this system seems to be a non-stable product of the metabolism, since it tends to disappear after growth maximum has been reached.

D. Production of Antibiotics (VII)

Oxford (1947) recorded that a strain of *M. virescens* produced an antibiotic substance, which was active against the Gram-positive *Staphylococcus aureus.* This observation inspired some experiments with *M. virescens* and *Ch. coralloides,* which were tested for antibiotic

production. The experiments are to be regarded as preliminary. It was first shown that the bacteriolytic substances do not act as antibiotic agents. In other experiments *M. virescens* was found to produce and secrete substances, which to a large extent inhibited the growth of the Gram-negative *Aerobacter aerogenes* but had no effect on the Gram-positive *Staphylococcus aureus*. In the case of *Ch. coralloides* a similar growth-inhibiting effect was visible on *Aerobacter aerogenes* and in addition a complete inhibition of the growth of *Staphylococcus aureus* was produced.

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